Supplementary Material

Committees, Leadership, and Investigators

Coalition COVID-19 Brazil

Coalition COVID-19 Brazil is a research alliance coordinated by the following institutions: Hospital Israelita Albert Einstein, HCor, Hospital Sírio Libanês, Hospital Moinhos de Vento, Hospital Alemão Oswaldo Cruz, A Beneficência Portuguesa de São Paulo, Brazilian Clinical Research Institute (BCRI) and Brazilian Research in Intensive Care Network – BRICNet.

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EFFICACY AND SAFETY OF TOCILIZUMAB IN PATIENTS WITH MODERATE TO SEVERE COVID-19 AND SEVERITY PREDICTORS: RANDOMIZED CONTROLLED CLINICAL TRIAL

Statistical Analysis Plan

Version: 1 – 2020 JULY 10 based on Protocol Version 2

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Introduction

Many studies have been conducted around the world to find a treatment for COVID-19. Some of these studies test the efficacy of different treatments in use for another diseases. The expectation is to mitigate the action of Sars-Cov-2, cause of the current pandemic, aiming to reduce the hospitalized patient's gravity and mortality.

The Coalition VI project's objective is to assess the efficacy and safety of interleukin-6 inhibitor Tocilizumab therapy for the treatment of hospitalized adult patients diagnosed with COVID-19. In this way, the study hypothesis to be investigated is that tocilizumab added to standard of care is superior to standard of care alone in moderate to severe cases of COVID-19 and severity predictors.

Objectives

Primary Objective and outcome measure

The primary objective of this trial is to assess the effect of tocilizumab combined with standard of care compared to only standard of care, according with the clinical status of patients 15 days after randomization. This classification will be measured by a 7-point ordinal scale as follows:

1) Patient not hospitalized, with no limitation in activities
2) Patient not hospitalized, with limitation in activities
3) Patient in the hospital, without supplemental oxygen
4) Patient in the hospital, with supplemental oxygen
5) Patient in the hospital on non-invasive positive pressure ventilation (NIPPV) or high flow nasal cannula
6) Patient on mechanical ventilation
7) Death

Secondary Objectives and outcomes measures

The secondary objectives are to assess the treatment effect with tocilizumab from some indicators of patient's clinical status evolution, including related measures of oxygen therapy, length of stay, adverse events, mortality, as described below:

1. All-cause mortality from randomization to day 28

2. Hospital mortality
3. Degree of organ dysfunction assessed by the Sequential Organ Failure Assessment (SOFA) score at day 8 and day 15 after randomization
4. Clinical status at days 8 and 29 after randomization, using the 7-level ordinal Scale
5. Ventilator free days within 29 days
6. Time until oxygen support independence within 29 days
7. Length of hospitalization
8. Incidence of secondary infections
9. Occurrence of thromboembolic events (stroke, myocardial infarction, deep vein thrombosis, pulmonary thromboembolism)
10. Incidence of adverse events

Secondary exploratory objectives

1. To assess the association of inflammatory markers and cytokines with clinical outcomes
2. To assess the kinetics of haemostatic parameters, inflammatory markers, cytokines, peripheral blood flow cytometry, complete blood count, renal and liver function tests
3. To assess viral clearance of SARS-CoV2 at D8

The following exploratory tests will be conducted and correlated with clinical outcomes:

- Biomarker measurement of D-dimer, CRP, LDH, ferritin, IL-6, TNFα, IL2 receptor (CD25), and IL-10.
- Coagulation studies PT/PTT, fibrinogen, vWF, ristocetin cofactor and factor 8.

Study population

Adult patients (Age ≥ 18 years old) hospitalized with moderate to severe COVID-19 who require supplemental oxygen, in several sites in Brazil. More details about the considered population are described in inclusion and exclusion criteria.

Inclusion criteria

1. Confirmed diagnosis of SARS-CoV-2 infection
2. Computed tomography (or chest X-ray) of the chest consistent with COVID-19
3. More than three days of symptoms related to COVID-19
4. 18 years or older;
5. Need for oxygen supplementation to maintain SpO_2 > 93% OR need for mechanical ventilation less than 24 hours before the randomization
6. Two or more of the following inflammatory tests:
    i. D-dimer > 1,000 ng/mL
    ii. C reactive protein (CRP) > 5 mg/dL
    iii. Ferritin > 300 mg/dL
    iv. Lactate dehydrogenase (LDH) > upper limit of normal

Exclusion Criteria

1. Need for mechanical ventilation for 24 hours or more before the randomization
2. Hypersensitivity to tocilizumab
3. Patients without therapeutic perspective or in palliative care
4. Active non-controlled infections (other than COVID-19)
5. Neutrophil count < 0.5 x 10^9/L
6. Platelet count < 50 x 10^9/L
7. Liver disease, cirrhosis or elevated AST or ALT above 5 times the upper limit of normal
8. Renal disease with estimate glomerular filtration below 30 mL/min/1.72 m^2 (MDRD or CKD-EPI scores)
9. Breastfeeding women
10. Pregnancy
11. Other clinical conditions that contraindicate tocilizumab, according to the attending physician

Design study

This study (TOCIBRAS) was developed according to SPIRIT guidelines and registered in clinicaltrials.gov as NCT04403685. TOCIBRAS trial is an open-label, parallel-group, superiority, multicentre, randomized controlled trial phase III study and analysis will follow the intention-to-treat principle.

Patients was randomly assigned to each group in 1:1 allocation using block randomization method with blocks size of 2, 4, 6 and 8, stratified by age (< 60 and ≥ 60 years) and gender.

Experimental arm:

In the experimental arm, Tocilizumab will be administered as a single intravenous infusion at 8 mg/kg/dose. The maximum dose is 800 mg. To allow for a homogenous dose rounding between participant sites, the following weight-based scale will be applied:

- < 50 kg – 8 mg/kg/dose
- from 50 to 56 kg – 400 mg/dose
- from 57 to 68 kg – 500 mg/dose
- from 69 to 81 kg – 600 mg/dose
- from 82 to 93 kg – 700 mg/dose
- ≥ 94 kg to 800 mg/dose (max dose)

Control arm:

Standard of care (Best supportive care), according to the local protocol.

Statistical Analysis

Basic principles of statistical analysis

Primary analysis will follow the intention-to-treat principle, including all randomized participants according to the group they were originally assigned, regardless of having received or not the allocated treatment.

The security analysis will be carried out in a security population, defined as all participants who received less than one medication dose compared to those who did not received the treatment.

Patient clinical and demographic characteristics as a function of treatment will be described according to the appropriate summary statistics (proportions for categoric data,

means and standard deviations or median and interquartile range for continuous variables) are shown in **Table 1**.

Analyses will be performed with R software (R Core Team, 2020).

Sample size calculation

Considering an ordinal outcome with seven stages to be evaluated at 15^{th} day after randomization with the probabilities 30%, 20%, 8%, 8%, 4%, 15%, 15% (Beigel et al., 2020), respectively for stages 1 to 7, under the model of proportional odds ratios for the accumulated probabilities for the outcomes levels, a sample of 75 cases per arm (total of 150 cases) has 80% power to detect an odds ratio of 0.44, with a 5% significance level. (Whitehead, 1993)

Primary outcome analysis

Comparisons between groups will be performed using proportional odds model for ordinal outcomes adjusted for stratification variables (age, as continuous variable and gender). Furthermore, binary cumulated outcomes will be assessed by logistic regression models also adjusted for the same variables.

If odds proportionality does not hold in the final analysis, we intent to switch primary outcome to a binomial endpoint collapsing categories 1 to 5 and 6 to 7 (alive versus dead or on mechanical ventilation).

The results will be presented as shown in the **Table 2**.

Secondary outcomes analysis

Length of hospitalization and days until oxygen support independence will be presented as means and standard deviations and will be compared between groups with means differences estimated through generalized linear model with Gamma, Normal or Poisson distribution, the most adequate for each case.

Ventilator free days within 29 days will be analyzed using generalized additive models with Beta-binomial distribution.

Analysis of patient's clinical outcomes at days 8 and 29 after randomization, using the ordinal scale, will be performed with similar models used for primary outcomes analysis: proportional odds models.

Hospital mortality and all-cause mortality, in case of complete follow-up for all patients, will be tested using logistic regression. Otherwise, Cox proportional hazard model will be used, and results will be presented as hazard ratio.

Comparisons between binary outcomes events (measures of incidence of secondary infections, incidence of adverse events, occurrence of thromboembolic events) will compared using logistic regression models.

Degree of organ dysfunction assessed by the Sequential Organ Failure Assessment (SOFA) score at day 8 and day 15 after randomization will be analyzed with mixed linear models considering random intercepts for subjects. The treatment effect will be assessed by means differences at days 8 and 15 with respective confidence intervals with 95%.

All secondary outcome models will be adjusted for age and gender and results shall be presented as **Table 2**.

Interim analysis

For this study is expected only one interim analysis. "An interim analysis will be performed when 50% of the planned accrual is reached (n=75). The interim analysis will be performed by an independent DMC who will analyze efficacy and safety data, rate of recruitment, adherence to the protocol, data quality, and follow-up loss. These data will be provided by the study coordinating site to the committee via a report. The Lan DeMets method and O'Brien Fleming thresholds will be applied as a pre-defined criterion for study interruption. If the committee recommends continued accrual, the study will go onto completion (n=150).

Missing data

Primary and secondary outcomes, if missing information, will be imputed by multivariate imputation methods by chained equations using *mice* package (van Buuren, 2011). We intent to use stratification variables (age and gender) and ordinal scale at baseline as predictors in the equations for the missed outcomes.

It is possible that the data base lock will happen before time to events variables were acquired (mortality, days until oxygen support independence, and length of stay). Specifically for those variables, survival and days until oxygen support independence will be censored, and length of stay truncated at data base lock date.

Subgroup analysis

Subgroup analysis for the primary results will be evaluate the treatment effect at following subgroups: age (< or ≥ 60 years), sex, types of comorbidities (cardiovascular, pulmonary, hepatic, renal, obesity, high blood pressure, cancer, diabetes), and altered inflammatory markers.

Subgroups will be tested by adding an interaction parameter with group and results will be presented like Table 3, with the addition of a Forest Plot.

It is possible, however, that some of the models did not fit well for the 7-level ordinal scale, giving the small sample size. In that case, subgroups analyses will be done collapsing categories 1 to 5 and 6 to 7 (alive versus dead or on mechanical ventilation) with logistic regression models.

Sensitivity analysis

It is planned a sensitivity analysis for primary and secondary outcomes only in subjects where the protocol has been applied accordingly (*per protocol analysis)*: defined as subjects that received less than one medication dose, if allocated in treatment group, and who haven't received any dose of study medication, if allocated in control group.

If multiple imputation of primary or secondary outcomes were necessary, a second sensitivity analysis will be performed in a complete-case analysis.

Adverse events and security analysis

The security analysis will be performed comparing patients that received less than one Tocilizumab dose and those that do not received Tocilizumab, independently to the group were patients were allocated.

Security outcomes include death until the day 28 of randomization and another serious adverse events (AE). Those events will be analyzed according univariate form and as composite clinical outcome. Methods of time measurement to the event will be used to death and composite clinical outcome. Each AE will be counted one time to each participant and classified by severity and relationship with COVID-19 or study intervention. AEs will be codified using a current version of Medical Dictionary for Regulatory Activities (MedDRA). AEs will be presented by system organ class, duration (days). Drug related serious adverse events will be highlighted in a table or a list.

Given the biologic characteristics of tocilizumab, the following AEs will be of specific interest and will be captured: Secondary Infections

- Anaemia
- Liver function test abnormalities
- Diverticulitis
- Herpes zoster
- Headache
- Haemorrhage
- Thromboembolic events
- Serious infusion-related toxicities

The relating AEs with treatment will be graded according to the most recent version of the Common Terminology Criteria for Adverse Events (CTCAE). That is, the events will be classified on a scale of 1 to 5 as described below:

GRADE 1 - Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

GRADE 2 - Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate activities of daily living.

GRADE 3 - Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting selfcare in the activities of daily living.

GRADE 4 - Life-threatening consequences; urgent intervention indicated.

GRADE 5 - Death related to Adverse Event.

Additional analyses

The number of days free of invasive mechanical ventilation during the 28-day follow-up distribution according to the group may be presented as **Figure 1**.

Time to events (survival) and Days until oxygen support independence within 29 days will be presented in Kaplan-Meier curves.

Finally, 6-level ordinal scores (combining both levels at home in just one level), will be described daily as suggested in **Figure 2**.

Figure 1 – Distribution of ventilator free days stratified by group.

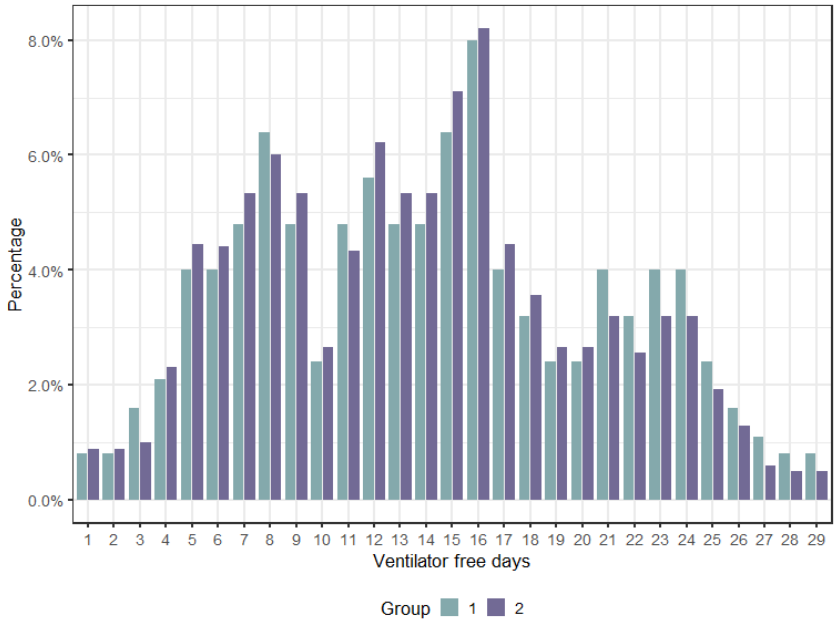
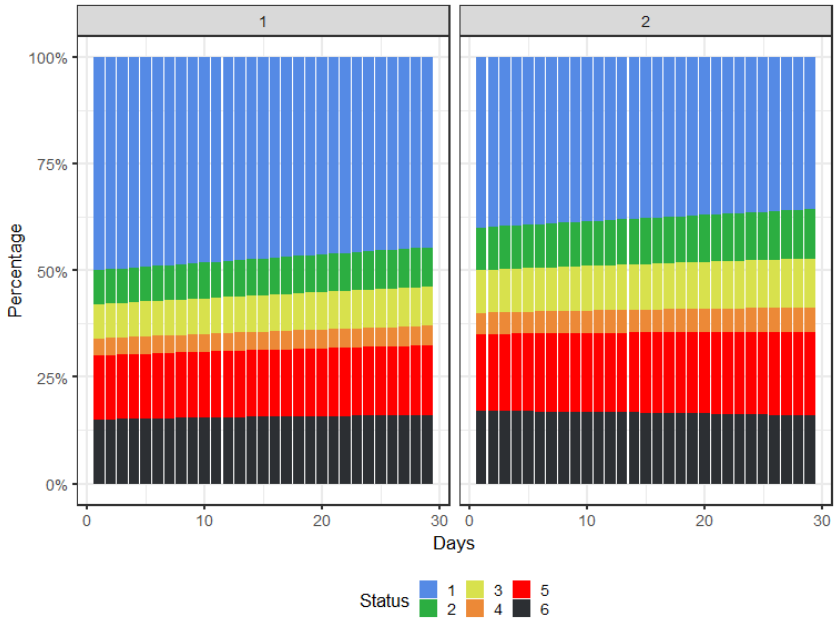


Figure 2 – Relative distribution of patient status, by number of days after randomization stratified by group.



Secondary exploratory objectives will be analyzed mainly with calculation of correlation coefficient (Pearson or Spearman, depends on the variables distribution) and illustrated by scatter plots. Occasionally, will be adjusted models with multiple explanatory variables.

Results presentation

Table 1 – Characteristics of patients and baseline clinical data.

	Tocilizumab (n = xx)	Control (n = xx)
Age (years), mean ± SD	xx.x ± xx.x	xx.x ± xx.x
Female sex, n (%)	x (xx.x)	x (xx.x)
Comorbidities, n (%)		
Hypertension	x (xx.x)	x (xx.x)
Diabetes	x (xx.x)	x (xx.x)
Obesity	x (xx.x)	x (xx.x)
AIDS	x (xx.x)	x (xx.x)
Heart failure	x (xx.x)	x (xx.x)
Previous myocardial infarction	x (xx.x)	x (xx.x)
Smoking (current or previous)	x (xx.x)	x (xx.x)
COPD	x (xx.x)	x (xx.x)
Asthma	x (xx.x)	x (xx.x)
Chronic kidney disease	x (xx.x)	x (xx.x)
Neuromuscular disease (including neurological sequelae)	x (xx.x)	x (xx.x)
Solid neoplasm	x (xx.x)	x (xx.x)
Hematological neoplasm	x (xx.x)	x (xx.x)
Medications, n (%)		
None	x (xx.x)	x (xx.x)
Corticosteroid (>5 mg prednisone for more than 30 days)	x (xx.x)	x (xx.x)
Other immunosuppressant	x (xx.x)	x (xx.x)
Hydroxychloroquine	x (xx.x)	x (xx.x)
Azithromycin	x (xx.x)	x (xx.x)
Remdesivir	x (xx.x)	x (xx.x)
Others	x (xx.x)	x (xx.x)
Vital signs, mean ± SD		
Glasgow Coma Scale	xx.x ± xx.x	xx.x ± xx.x
Systolic blood pressure (mmHg)	xx.x ± xx.x	xx.x ± xx.x
Diastolic blood pressure (mmHg)	xx.x ± xx.x	xx.x ± xx.x
Heart rate (bpm)	xx.x ± xx.x	xx.x ± xx.x
Respiratory rate (bpm)	xx.x ± xx.x	xx.x ± xx.x
Non-invasive O2 flow (L/min)	xx.x ± xx.x	xx.x ± xx.x
FIO2 (%)	xx.x ± xx.x	xx.x ± xx.x
PEEP (cmH2O)	xx.x ± xx.x	xx.x ± xx.x
Peripheral oxygen saturation (%)	xx.x ± xx.x	xx.x ± xx.x
Vasopressor, n (%)		
No	x (xx.x)	x (xx.x)
Norepinephrine ≤ 0.1 mcg/kg/min	x (xx.x)	x (xx.x)
Norepinephrine > 0.1 mcg/kg/min	x (xx.x)	x (xx.x)
Respiratory support, n (%)		

	Tocilizumab (n = xx)	Control (n = xx)
Oxygen Catheter	x (xx.x)	x (xx.x)
Venturi mask	x (xx.x)	x (xx.x)
CNAF	x (xx.x)	x (xx.x)
Mechanical ventilation	x (xx.x)	x (xx.x)
Laboratorial		
PAO2 (mmHg), median [IQR]	x.xx [x.xx - x.xx]	x.xx [x.xx - x.xx]
Hemoglobin, mean ± SD	x (xx.x)	x (xx.x)
Leukocytes (/uL), median [IQR]	x.xx [x.xx - x.xx]	x.xx [x.xx - x.xx]
Neutrophils (/uL)	x (xx.x)	x (xx.x)
Lymphocytes (/uL), median [IQR]	x.xx [x.xx - x.xx]	x.xx [x.xx - x.xx]
Platelets (/uL), mean ± SD	x (xx.x)	x (xx.x)
Creatinine (mg/dL)	x (xx.x)	x (xx.x)
Urea (mg/dL)	x (xx.x)	x (xx.x)
AST (U/L)	x (xx.x)	x (xx.x)
ALT (U/L)	x (xx.x)	x (xx.x)
Bilirubin (mg/dL)	x (xx.x)	x (xx.x)
D-Dimer (ng/ mL FEU)	x (xx.x)	x (xx.x)
CRP (mg/dL)	x (xx.x)	x (xx.x)
Ferritin (ug/L)	x (xx.x)	x (xx.x)
LDH (U/L)	x (xx.x)	x (xx.x)
Lactate (mg/dL), mean ± SD	x (xx.x)	x (xx.x)
Prothrombin time (INR)	x (xx.x)	x (xx.x)
aPTT	x (xx.x)	x (xx.x)
Troponin (ng/mL), median [IQR]	x.xx [x.xx - x.xx]	x.xx [x.xx - x.xx]
Beta-HCG, n (%)		
Negative	x (xx.x)	x (xx.x)
Positive	x (xx.x)	x (xx.x)
Unavailable	x (xx.x)	x (xx.x)
Anti-HbC total, n (%)		
Negative	x (xx.x)	x (xx.x)
Positive	x (xx.x)	x (xx.x)
Unavailable	x (xx.x)	x (xx.x)
HbSAg, n (%)		
Negative	x (xx.x)	x (xx.x)
Positive	x (xx.x)	x (xx.x)
Unavailable	x (xx.x)	x (xx.x)
Anti-HCV, n (%)		
Negative	x (xx.x)	x (xx.x)
Positive	x (xx.x)	x (xx.x)

	Tocilizumab (n = xx)	Control (n = xx)
Unavailable	x (xx.x)	x (xx.x)
Anti-HIV, n (%)		
Negative	x (xx.x)	x (xx.x)
Positive	x (xx.x)	x (xx.x)
Unavailable	x (xx.x)	x (xx.x)
Initial prescription		
Heparine, n (%)	x (xx.x)	x (xx.x)
Prophylactic	x (xx.x)	x (xx.x)
Therapeutic	x (xx.x)	x (xx.x)
Corticosteroid, n (%)		
No	x (xx.x)	x (xx.x)
Prednisone equivalent < 0.5 mg/kg/day	x (xx.x)	x (xx.x)
Prednisone equivalent ≥ 0.5 e < 1.0 mg/kg/day	x (xx.x)	x (xx.x)
Prednisone equivalent ≥ 1 mg/kg/day	x (xx.x)	x (xx.x)

Table 2 – Primary and secondary outcomes.

	Tocilizumab (n = xx)	**Control** (n = xx)	**Effect type**	**Effect size (CI 95%)**
Primary outcome				
Clinical status (7-level ordinal scale) at day 15, median [IQR]	x.xx [x.xx - x.xx]	x.xx [x.xx - x.xx]	Odds ratio	xx.x (xx.x – xx.x)
Secondary Outcomes				
All-cause mortality from randomization to day 28, n (%)	x (x.xx)	x (x.xx)	Odds ratio	xx.x (xx.x – xx.x)
Hospital mortality, n (%)	x (x.xx)	x (x.xx)	Odds ratio	xx.x (xx.x – xx.x)
SOFA, score at day 8, median [IQR]	x.xx [x.xx - x.xx]	x.xx [x.xx - x.xx]	Means difference	xx.x (xx.x – xx.x)
SOFA, score at day 15, median [IQR]	x.xx [x.xx - x.xx]	x.xx [x.xx - x.xx]	Means difference	xx.x (xx.x – xx.x)
Clinical status (7-level ordinal scale) at day 8, median [IQR]	x.xx [x.xx - x.xx]	x.xx [x.xx - x.xx]	Odds ratio	xx.x (xx.x – xx.x)
Clinical status (7-level ordinal scale) at day 29, median [IQR]	x.xx [x.xx - x.xx]	x.xx [x.xx - x.xx]	Odds ratio	xx.x (xx.x – xx.x)
Ventilator free days within 29 days, mean ± SD	xx.x ± xx.x	xx.x ± xx.x	Rate ratio	xx.x (xx.x – xx.x)
Days until oxygen support independence within 29 days, mean ± SD	xx.x ± xx.x	xx.x ± xx.x	Rate ratio	xx.x (xx.x – xx.x)
Length of hospitalization (days), mean ± SD	xx.x ± xx.x	xx.x ± xx.x	Rate ratio	xx.x (xx.x – xx.x)
Incidence of secondary infections, n (%)	x (x.xx)	x (x.xx)	Odds ratio	xx.x (xx.x – xx.x)
Occurrence of thromboembolic events (stroke, myocardial infarction, deep vein thrombosis, pulmonary thromboembolism), n (%)	x (x.xx)	x (x.xx)	Odds ratio	xx.x (xx.x – xx.x)
Incidence of adverse events, n (%)	x (x.xx)	x (x.xx)	Odds ratio	xx.x (xx.x – xx.x)

Table 3 – Subgroups analysis.

Model	Tocilizumab (n = xx)	Control (n = xx)	Effect size measure (CI 95%)	p-value	Interaction p-value
Sex					
Male	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
Female	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Age					
< 60 years	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
≥ 60 years	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Cardiovascular disease					
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Pulmonary disease					
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Liver disease					
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Immunosuppression					
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Obesity					
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Hypertension					
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Solid neoplasm					
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Diabetes					
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Inflammatory markers					
LDH > upper limit of normal					
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
CRP > 5 mg/dL					
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	

Model	Tocilizumab (n = xx)	Control (n = xx)	Effect size measure (CI 95%)	p-value	Interaction p-value
D-Dimer > 1.000 ng/mL FEU					
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	
Ferritin > 300 ug/dL					
No	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	x.xxx
Yes	x (xx.x)	x (xx.x)	xx.x (xx.x – xx.x)	x.xxx	

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Data Monitoring Committee Letter

De: PEDRO POVOA <pedrorpovoa@gmail.com>
Enviada em: quarta-feira, 22 de julho de 2020 04:32
Para: Viviane Cordeiro Veiga <viviane.veiga@bp.org.br>; Alexandre Biasi <abiasi@hcor.com.br>
Cc: Rodrigo T. Calado <rtcalado@fmrp.usp.br>; Armando Teixeira-Pinto <armando.teixeira-pinto@sydney.edu.au>
Assunto: DSMB letter

Safety and Efficacy of Tocilizumab in Moderate to Severe COVID-19 With Inflammatory Markers (TOCIBRAS) (ClinicalTrials.gov Identifier: NCT04403685)

The DMC members met on two occasions to analyse the interim results after receiving the data from the investigators: on July 17th and July 21st. The members analysed and discussed the results and were unanimous in their conclusion.

Based on the interim results, the DMC deliberated that there were enough safety concerns, and most likely futility, to justify the suspension of the trial's recruitment. After the accrual of 129 patients (with 84 having completed the follow up at the time of the last meeting), eight patients (18%) died before day 15 in the intervention arm (Tocilizumab), while the control group presented 2 deaths (4.5%). This difference is statistically significant (p=0.042) for a significance level of 0.05, although the test is not adjusted for the interim analysis. Given that mortality is part of the composite primary outcome, it is therefore unlikely that the follow up of already included patients, as well as the recruitment of additional patients, could invert the association and show superiority of the intervention arm. The final conclusion of the potential harm of Tocilizumab, should be made after analysing the completion of the follow up of the remaining 45 patients who completed the assigned treatment but are still being followed-up for the primary endpoint.

Additionally, there was higher incidence of adverse events (56% vs 38%) for the Tocilizumab arm and no evidence of benefits in the secondary outcomes.

Armando T Pinto

Rodrigo T Calado

Pedro Póvoa

Methods

Additional Details on Trial Design and Oversight

The trial registration on Clinicaltrials.gov was finalised only after enrolment of the first patient because of an administrative error by the research team. On May 8th, an eligible patient was identified at our centre and enrolment offered to the patient. At the same day, the protocol was included in ClinicalTrials.gov but could not be registered. On May 11th, we received a response with a modified Protocol Registration and Results System for registration. On May 12th, we uploaded our protocol information in ClinicalTrials.gov as approved by the Brazilian Ethics authorities. As we did not receive a reply from ClinicalTrials.gov in subsequent days, a new contact was made on May 24th and the protocol as initially submitted was published.

Additional Details on Patients

In the first version of the trial protocol, need of mechanical ventilation was an exclusion criterion. On June 4th, 2020, after the study was initiated, an amendment was made to allow inclusion of patients under mechanical ventilation for less than 24 hours. On July 7th, 2020 chest X-ray evidence of COVID-19 was included as an alternative to computed tomography in the inclusion criteria. Below are the original inclusion/exclusion and the final inclusion/exclusion reflecting this change.

Inclusion criteria (Initial):

1. Confirmed diagnosis of SARS-CoV-2 infection
2. Computed tomography of the chest consistent with COVID-19
3. More than three days of symptoms related to COVID-19
4. 18 years of age or older
5. Need for oxygen supplementation to maintain SpO_2 > 93%
6. Two or more of the following inflammatory tests:
    - D-dimer > 1,000 ng/mL
    - C reactive protein (CRP) > 5 mg/dL
    - Ferritin > 300 mg/dL
    - Lactate dehydrogenase (LDH) > upper limit of normal

Exclusion criteria (initial):

1. Need for mechanical ventilation
2. Hypersensitivity to tocilizumab
3. Patients without therapeutic perspective or under palliative care
4. Active non-controlled infections (other than COVID-19)
5. Neutrophil count < 0.5 x 10^9/L
6. Platelet count < 50 x 10^9/L
7. Liver disease, cirrhosis or elevated AST or ALT above 5 times the upper limit of normal
8. Renal disease with estimate glomerular filtration below 30 mL/min/1.72 m^2 (MDRD or CKD-EPI scores)

9. Breastfeeding
10. Pregnancy
11. Other clinical conditions that contraindicate tocilizumab, according to the attending physician

Inclusion criteria (final):

1. Confirmed diagnosis of SARS-CoV-2 infection
2. Computed tomography (or chest X-ray) of the chest consistent with COVID-19
3. More than three days of symptoms related to COVID-19
4. 18 years of age or older
5. Need for oxygen supplementation to maintain SpO_2 > 93% OR or were under mechanical ventilation for less than 24 hours before randomization.
6. Two or more of the following inflammatory tests:
    i. D-dimer > 1,000 ng/mL
    ii. C reactive protein (CRP) > 5 mg/dL
    iii. Ferritin > 300 mg/dL
    iv. Lactate dehydrogenase (LDH) > upper limit of normal

Exclusion criteria (final):

1. Need for mechanical ventilation for more than 24 hours before the randomization
2. Hypersensitivity to tocilizumab
3. Patients without therapeutic perspective or in palliative care
4. Active non-controlled infections (other than COVID-19)

5. Neutrophil count < 0.5 x 10^9/L
6. Platelet count < 50 x 10^9/L
7. Liver disease (cirrhosis) or elevated AST or ALT above 5 times the upper limit of normal
8. Renal disease with estimate glomerular filtration below 30 mL/min/1.72 m^2 (MDRD or CKD-EPI scores)
9. Breastfeeding
10. Pregnancy
11. Other clinical conditions that contraindicate tocilizumab, according to the attending physician

Additional Details on Interventions

Both groups received the current standard care treatment for COVID-19, which included daily monitoring with clinical assessment by the attending physician, routine laboratory tests (blood count, urea, creatinine, liver enzymes and bilirubin) at the discretion of the attending physician, respiratory therapy, surveillance of vital parameters according to the patient's location (inpatient unit or ICU) at least once per period, addition of ventilatory support measures, as recommended by the attending physician, prophylaxis of stress ulcers (if indicated) and venous thromboembolism according to the protocol of each institution and addition of other therapies such as antibiotics, corticosteroids, antivirals (e.g., oseltamivir for suspected influenza coinfection).

Additional Details on Outcomes

6-Level Ordinal Scale

Clinical status at 8 days was assessed using a 6-level ordinal scale, defined as follows: 1, not hospitalized; 2, hospitalized and not using supplemental oxygen; 3, hospitalized and using supplemental oxygen; 4, hospitalized and using oxygen supplementation via high-flow nasal cannula or non-invasive ventilation; 5, hospitalized and on mechanical ventilation; 6, death.

Exploratory Outcomes

The following exploratory tests were conducted and were analysed over time and compared between groups

- Biomarker measurement of D-dimer, CRP, LDH, ferritin, IL-6, TNFα, IL2 receptor (CD25), and IL-10
- Coagulation studies PT/PTT, fibrinogen, vWF, ristocetin cofactor and factor 8.

The measurement of the cytokines IL-6, TNFα, IL-10, as well as the IL-2 receptor (CD25), was performed by capture ELISA system. Briefly, the serum samples were incubated in an appropriate dilution in polystyrene plates pre-coated as monoclonal antibodies against the cytokine of interest for 30 minutes. After washing, there were incubated with peroxidase-labelled monoclonal antibody for 30 minutes. After a new wash, an additional incubation with 3.3, 5.5'-tetramethylbenzidine (TMB) and hydrogen

peroxide (H_2O_2) was performed. After 10 minutes, the reaction was stopped by adding 1N H_2SO_4 and each well will be evaluated by spectrophotometry at wavelength 450nm.

In relation to the coagulation tests the following steps were performed. All samples for coagulation tests were collected in tubes containing citrate 3.2% as anticoagulant. Tubes were centrifuged at 2.200 g and plasma was aliquoted and stored at -80° C. For analysis, samples were thawed at 37° C for 20 minutes. All assays were performed on ACL TOP 750 analyser (Instrument Laboratories, Bedford, USA) accordingly to standard protocols. The PT will be performed using Hemosil® RecombiPlasntin 2G; PTT and factor 8 assays will be performed using Hemosil® Synthasil and Hemosil® Factor VIII deficient plasma; Factor VIII assay will be performed using a single-point assay (1/20 dilution in buffer). Fibrinogen will be performed using Hemosil® QFA Thrombin (Bovine) reagent by Clauss method. Von Willebrand - Hemosil® VWF assays will be performed using Hemosil® VWF:Ag and ristocetin cofactor assay will be performed using Hemosil® VWF-Rco, all immunoturbidimetric tests. All reagents are from Instrument Laboratories (IL, Bedford, USA).

Additional Details on Statistical Analysis

Statisticians were aware of treatment assignment when they analysed data. Pre-specified subgroup analyses were conducted with interaction terms for group and the following variables: age (<60 years and ≥60), gender, comorbidities (cardiovascular, pulmonary, hepatic, renal, obesity, high blood pressure, cancer, diabetes), and serum levels of lactate dehydrogenase higher than upper limit of normal (yes vs. no), c-reactive protein higher

than 5 mg/dL (yes vs. no), D-dimer higher than 1,000 ng/mL FEU (yes vs. no), and ferritin higher than 300 mg/dL (yes vs. no).

Table S1. List of sites and number of randomized patients per site

Site	Number of patients randomized
A Beneficência Portuguesa de São Paulo	68
HCor	27
Hospital Alemão Oswaldo Cruz – Unidade Paulista	10
Hospital São Paulo - UNIFESP	9
Hospital SEPACO	5
Hospital Alemão Oswaldo Cruz – Unidade Vergueiro	4
Hospital Sírio Libanês	3
Hospital Israelita Albert Einstein	2
Hospital Santa Paula	1

Table S2. Additional characteristics of the population at baseline

	Tocilizumab (n = 65)	**Control** (n = 64)
Vital signs		
Glasgow Coma Scale, median [IQR]	15 [15-15] (n = 63)	15 [15-15] (n = 62)
Systolic blood pressure (mmHg), mean ± SD	122 ± 18	125 ± 19
Diastolic blood pressure (mmHg), mean ± SD	74 ± 12	75 ± 11
Heart rate (bpm), mean ± SD	83 ± 17	84 ± 18
Laboratorial		
Haemoglobin, mean ± SD	12.8 ± 1.8	13.2 ± 1.6
Leukocytes (/uL), $10^3/mm^3$, mean ± SD	8.8 ± 4.9	8.5 ± 4.1
Neutrophils (/uL), $10^3/mm^3$, mean ± SD	7.1 ± 4.8 (n = 64)	7.0 ± 3.9
Lymphocytes (/uL), mean ± SD	968.9 ± 675.4	971.5 ± 521.0
Platelets (/uL), $10^3/mm^3$, mean ± SD	219.5 ± 71.1	219.7 ± 73.6
Creatinine (mg/dL), mean ± SD	1.1 ± 0.4	1.0 ± 0.3
Urea (mg/dL), mean ± SD	42.5 ± 22.2	40.2 ± 23.4
AST (U/L), median [IQR]	45 [30-56] (n = 56)	42 [30-60] (n = 58)
ALT (U/L), median [IQR]	42 [26-60] (n = 58)	43 [25-58] (n = 59)
Bilirubin (mg/dL), mean ± SD	0.5 ± 0.3 (n = 46)	0.5 ± 0.3 (n = 50)
Lactate (mg/dL), mean ± SD	14.9 ± 6.5 (n = 46)	15.0 ± 7.9 (n = 50)
Prothrombin time (INR), median [IQR]	1.1 [1.0-1.2] (n = 54)	1.1 [1.1- 1.2] (n = 60)
aPTT, median [IQR]	1.0 [0.9-1.1] (n = 53)	1.1 [1.0-1.3] (n = 59)

IQR= interquartile range; SD, standard deviation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; aPTT, activated partial thrombin

Table S3. Use of other medications in the first 15 days

Medication	Tocilizumab (n = 67)	Control (n = 62)	Effect size measure (CI 95%)	p-value
Antibiotic	64 (95.5)	61 (98.4)	0.35 (0.04 - 3.43)	0.365
Antiviral	7 (10.4)	3 (4.8)	2.25 (0.55 - 9.18)	0.257
Corticosteroid	56 (83.6)	55 (88.7)	0.66 (0.24 - 1.83)	0.423

Table S4. Primary outcome – cumulative proportions*

Cumulative proportions of 7-level ordinal scale at day 15	Tocilizumab (n = 65)	Control (n = 64)	Effect size (CI 95%)
Category 7, n (%)	11 (16.9)	2 (3.1)	6.42 (1.59 to 43.2)
Categories 6-7, n (%)	18 (27.7)	13 (20.3)	1.54 (0.66 to 3.66)
Categories 5-7, n (%)	18 (27.7)	17 (26.6)	1.04 (0.46 to 2.37)
Categories 4-7, n (%)	24 (36.9)	27 (42.2)	0.78 (0.36 to 1.65)
Categories 3-7, n (%)	30 (46.2)	33 (51.6)	0.79 (0.37 to 1.65)
Categories 2-7, n (%)	33 (50.8)	38 (59.4)	0.69 (0.33 to 1.43)

CI=confidence interval

*The assumption of odds proportionality foes not hold (Brand test, p-value=0.039), so we change primary outcome to a binomial endpoint collapsing categories 1 to 5 and 6 to 7 (alive versus dead or no mechanical ventilation)

Table S5. Main analysis and sensitivity analysis for the primary outcome

	Odds ratio (95% CI)
Main analysis (adjusted for stratification variables age and sex)	1.54 (0.66 to 3.66)
Per protocol (only patients who were treated as assigned) *	1.48 (0.63 to 3.53)
Post hoc analysis adjusted for baseline level of respiratory support, age and gender	2.62 (0.96 to 7.61)

*This analysis excludes two patients from the Control Group who received tocilizumab. The two patients in the control group who received tocilizumab were still in hospital and needing supplemental oxygen at day 15 (level 4 of the 7-level ordinal scale). Both patients were discharged alive from hospital and were at home without limitations at day 29.

Table S6. Subgroups analysis for the primary outcome

	Tocilizumab (n = 65)	Control (n = 64)	Odds Ratio (95% CI)
Total, n (%)	18 (27.7)	13 (20.3)	1.54 (0.66 to 3.66)
Sex			
Male	14 (31.8)	11 (25.0)	1.42 (0.54 to 3.72)
Female	4 (19.0)	2 (10.0)	2.04 (0.32 to 13.0)
Age			
< 60 years	7 (19.4)	4 (11.1)	1.95 (0.51 to 7.4)
≥ 60 years	11 (37.9)	9 (32.1)	1.32 (0.44 to3.97)
Cardiovascular disease			
Yes	2 (28.6)	3 (60.0)	0.15 (0.01 to 1.95)
No	16 (27.6)	10 (16.9)	2.11 (0.83 to 5.37)
Pulmonary disease			
Yes	3 (33.3)	0 (0.0)	16.9 (0.48 to 597.54)
No	15 (26.8)	13 (22.4)	1.16 (0.48 to 2.81)
Chronic kidney disease			
Yes	4 (80.0)	0 (0.0)	-
No	14 (23.3)	13 (20.6)	1.17 (0.47 to2.88)
Obesity			
Yes	5 (33.3)	2 (12.5)	2.39 (0.36 to 15.87)
No	13 (26.0)	11 (22.9)	1.35 (0.52 to 3.56)
Hypertension			
Yes	10 (33.3)	9 (26.5)	1.54 (0.51 to4.67)
No	8 (22.9)	4 (13.3)	1.68 (0.43 to 6.63)
Solid neoplasm			
Yes	2 (50.0)	1 (20.0)	1.65 (0.08 to 35.61)
No	16 (26.2)	12 (20.3)	1.52 (0.63 to 3.71)
Diabetes			
Yes	6 (27.3)	7 (35.0)	0.60 (0.25 to 2.35)
No	12 (27.9)	6 (13.6)	2.89 (0.92 to 9.12)
Inflammatory markers			
LDH > upper limit of normal			
No	2 (16.7)	1 (10.0)	1.41 (0.10 to 19.33)
Yes	15 (28.8)	12 (22.2)	1.59 (0.63 to 4.02)
CRP > 5 mg/dL			
No	2 (25.0)	0 (0.0)	3.65 (0.09 to 152.25)
Yes	16 (28.1)	13 (21.7)	1.40 (0.59 to 3.33)
D-Dimer > 1.000 ng/mL FEU			
No	7 (23.3)	4 (9.5)	3.36 (0.85 to 13.3)
Yes	11 (31.4)	9 (40.9)	0.66 (0.21 to 2.10)
Ferritin > 300 ug/dL			
No	8 (26.7)	5 (18.5)	1.90 (0.50 to7.25)
Yes	10 (28.6)	8 (22.2)	1.23 (0.40 to 3.79)

CI=confidence interval; LDH=lactate dehydrogenase; CRP=C-reactive protein

Table S7. Adjudicated causes of in-hospital deaths

Causes of in-hospital death	Tocilizumab (n = 14)	Control (n=6)
Covid-19 related acute respiratory failure or multiple organ dysfunction	14	5
Covid-19 and cerebral haemorrhage	0	1

Table S8. Adjudicated causes of death at day 28 compared with treatment group, age and clinical status on 7-level ordinal scale

Age	Treatment	Clinical status on seven level ordinal scale (baseline)*	Adjudicated cause of death at day 28
89	Tocilizumab	4	Covid-19 related acute respiratory failure or multiple organ dysfunction
54	Tocilizumab	5	Covid-19 related acute respiratory failure or multiple organ dysfunction
61	Tocilizumab	6	Covid-19 related acute respiratory failure or multiple organ dysfunction
73	Tocilizumab	6	Covid-19 related acute respiratory failure or multiple organ dysfunction
72	Tocilizumab	4	Covid-19 related acute respiratory failure or multiple organ dysfunction
72	Tocilizumab	6	Covid-19 related acute respiratory failure or multiple organ dysfunction
56	Tocilizumab	6	Covid-19 related acute respiratory failure or multiple organ dysfunction
81	Tocilizumab	6	Covid-19 related acute respiratory failure or multiple organ dysfunction
84	Tocilizumab	5	Covid-19 related acute respiratory failure or multiple organ dysfunction
63	Tocilizumab	6	Covid-19 related acute respiratory failure or multiple organ dysfunction
43	Tocilizumab	6	Covid-19 related acute respiratory failure or multiple organ dysfunction
47	Tocilizumab	6	Covid-19 related acute respiratory failure or multiple organ dysfunction
71	Tocilizumab	5	Covid-19 related acute respiratory failure or multiple organ dysfunction
43	Tocilizumab	4	Covid-19 related acute respiratory failure or multiple organ dysfunction
62	Control	4	Covid-19 related acute respiratory failure or multiple organ dysfunction
66	Control	4	Covid-19 related acute respiratory failure or multiple organ dysfunction
54	Control	6	Covid-19 and cerebral haemorrhage
71	Control	6	Covid-19 related acute respiratory failure or multiple organ dysfunction
77	Control	4	Covid-19 related acute respiratory failure or multiple organ dysfunction
70	Control	6	Covid-19 related acute respiratory failure or multiple organ dysfunction

* **Clinical status on seven level ordinal scale (baseline):** 4: hospitalised and receiving supplemental oxygen; level 5: hospitalised and using non-invasive positive pressure ventilation or high-flow nasal cannula; level 6: hospitalised and receiving mechanical ventilation

Table S9. Biomarker measurements analyses

	Tocilizumab (n = 65)	Control (n = 64)	Effect size Rate ratio (CI 95%)
IFN-y (pg/mL), mean (SD)			
baseline	12 ± 19 (n = 44)	11 ± 16 (n = 43)	1.07 (0.54 to 2.09)
D5	15 ± 28 (n = 28)	3 ± 5 (n = 30)	3.64 (1.67 to 7.96)
D8	14 ± 15 (n = 14)	17 ± 18 (n = 20)	0.92 (0.35 to 2.44)
IL-17A (pg/mL), mean (SD)			
baseline	56 ± 57 (n = 44)	48 ± 51 (n = 43)	1.18 (0.74 to 1.87)
D5	33 ± 37 (n = 28)	27 ± 29 (n = 30)	1.10 (0.63 to 1.93)
D8	35 ± 37 (n = 14)	41 ± 42 (n = 20)	0.86 (0.41 to 1.79)
TNF (pg/mL), mean (SD)			
Baseline	5.4 ± 7.7 (n = 44)	4.1 ± 1.9 (n = 43)	1.09 (0.82 to 1.46)
Day 5	3.7 ± 2.5 (n = 28)	5.1 ± 6.2 (n = 30)	0.85 (0.60 to 1.20)
Day 8	3.4 ± 1.9 (n = 14)	5.1 ± 3.0 (n = 20)	0.71 (0.45 to 1.11)
IL-10 (pg/mL), mean (SD)			
baseline	18 ± 11 (n = 44)	13 ± 7 (n = 43)	1.39 (1.00 to 1.92)
D5	6 ± 3 (n = 28)	7 ± 4 (n = 30)	0.91 (0.61 to 1.35)
D8	33 ± 58 (n = 14)	9 ± 8 (n = 20)	2.59 (1.54 to 4.34)
IL-6 (pg/mL), mean (SD)			
baseline	192 ± 313 (n = 44)	208 ± 586 (n = 43)	1.28 (0.72 to 2.29)
D5	761 ± 1362 (n = 28)	38 ± 56 (n = 30)	12.47 (6.28 to 24.77)
D8	1534 ± 1900 (n = 14)	136 ± 326 (n = 20)	20.27 (8.48 to 48.45)
IL-4 (pg/mL), mean (SD)			
baseline	12 ± 27 (n = 44)	8 ± 12 (n = 43)	1.32 (0.73 to 2.37)
D5	10 ± 17 (n = 28)	5 ± 6 (n = 30)	1.68 (0.85 to 3.29)
D8	8 ± 8 (n = 14)	17 ± 35 (n = 20)	0.67 (0.29 to 1.54)
IL-2 (pg/mL), mean (SD)			
baseline	3 ± 3 (n = 44)	2 ± 1 (n = 43)	1.21 (0.90 to 1.62)
D5	2 ± 1 (n = 28)	2 ± 2 (n = 30)	0.83 (0.59 to 1.18)
D8	3 ± 1 (n = 14)	3 ± 2 (n = 20)	1.02 (0.65 to 1.60)
PCR (mg/dL), mean (SD)			
baseline	16 ± 10 (n = 63)	19 ± 28 (n = 63)	0.98 (0.66 to 1.46)
D5	5 ± 11 (n = 55)	13 ± 20 (n = 54)	0.31 (0.2 to0.47)
D8	4 ± 11 (n = 35)	9 ± 13 (n = 48)	0.19 (0.12 to 0.31)

IC=confidence interval; IFN=interferon; SD=standard deviation; IL=interleukin; TNF=tumour necrosis factor; PCR=C-reactive protein

Table S10. Effect of tocilizumab on the primary outcome, length of hospital stay and 15-day death according to duration of symptoms at randomization

	Tocilizumab	Control	Effect measure	
	(n = 65)	(n = 64)	Measure	IC[95%]
Primary outcome (ordinal 6 or 7)				
Days from symptoms onset to randomization				
< 10 days	9/29 (31%)	7/30 (23.3%)	OR*	1.48 [0.47 to 4.69]
≥ 10 days	9/36 (25%)	6/34 (17.6%)	OR*	1.56 [0.49 to 4.96]
Death within 15 days				
Days from symptoms onset to randomization				
< 10 days	5/29 (17.2%)	2/30 (6.7%)	OR†	2.56 [0.51 to 12.89]
≥ 10 days	6/36 (16.7%)	0/34 (0%)	OR†	-
Length of hospital stay				
Days from symptoms onset to randomization				
< 10 days	11.8 ± 7.4 (n=29)	14.7 ± 8.4 (n=30)	MD‡	-2.9 [-7.2 to 1.3]
≥ 10 days	10.9 ± 8.6 (n=36)	14.7 ± 8.3 (n=34)	MD‡	-3.8 [-7.7 to 0.1]

* Odds ratio from Logistic regression

†Odds ratio from Logistic regression with correction (Firth, D. (1993). Bias reduction of maximum likelihood estimates. Biometrika 80, 27–38.)

‡Mean difference from linear regression model

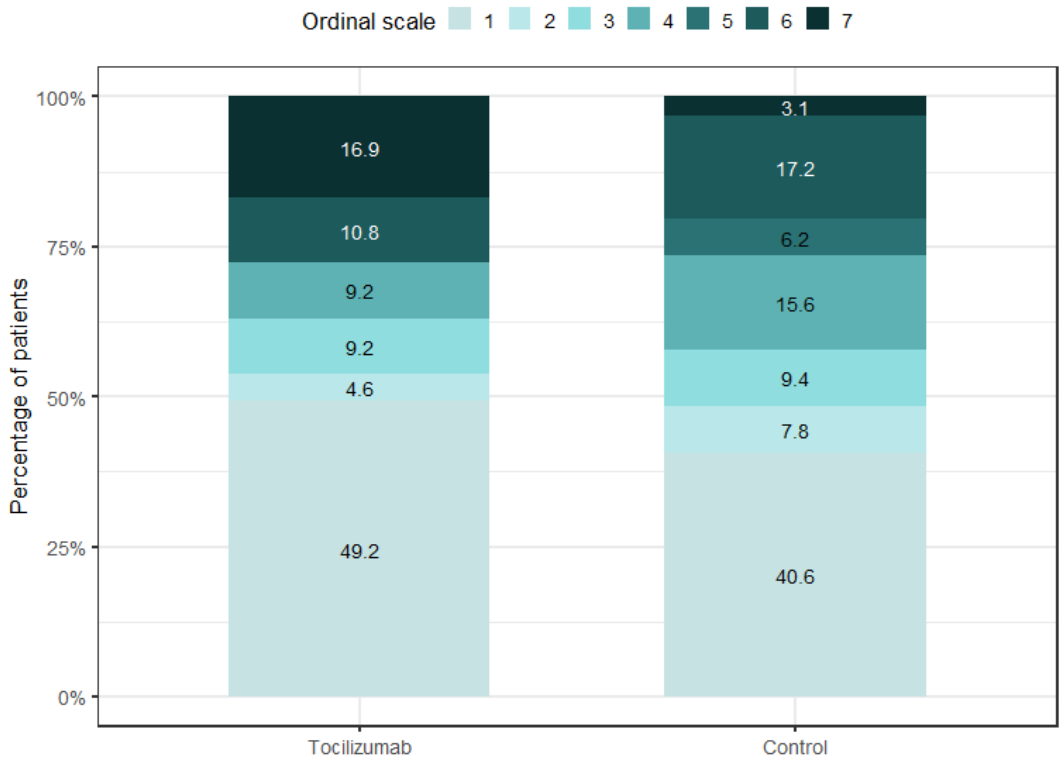

Figure S1- Relative distribution of patient status at day 15, stratified by group - Scores in the scale means: 1-Not hospitalized with no limitation on activities; 2-Not hospitalized but with limitation on activities; 3-Hospitalized, not receiving supplemental oxygen; 4-Hospitalized, receiving supplemental oxygen; 5-Hospitalized, receiving non-invasive ventilation or high-flow nasal cannula; 6-Patient on mechanical ventilation; 7-Death.

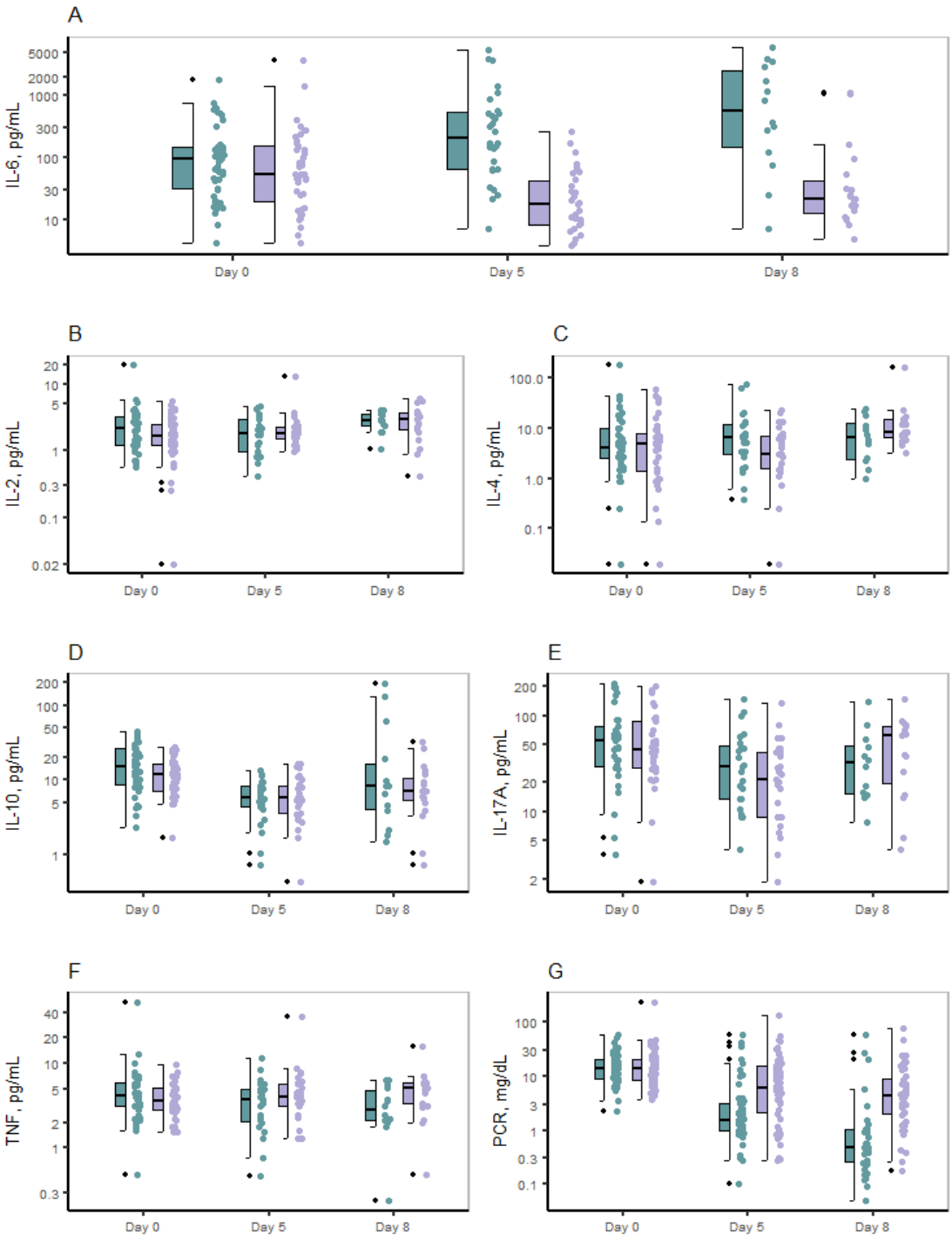

Figure S2- Serum inflammatory markers and cytokines at baseline, D5 and D8 in the tocilizumab (green) and control groups (purple)